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(54) Title: DHEA COMBINATION THERAPY

(57) Abstract

There are provided medicaments, methods of making them, and kits, which include (1) a 17-ketosteroid compound and/or (2) anti-serum either poly- or monoclonal to Interleukin 10, Interleukin 2 or Interleukin 12, or with any compound which can effectively inhibit synthesis or the biological function of Interleukin 10, Interleukin 12 or Interleukin 2, or with an Interleukin 10, Interleukin 12, or Interleukin 2 receptor molecule blocking agent, or with anti-serum, either polyclonal or monoclonal to human alpha-fetoprotein. There are also provided methods of treatment involving such compounds or combinations of compounds, including enhancing Th_1 immune protective response when using the 17-ketosteroid compound as an anti-viral, anti-bacterial, anti-mycoplasm or anti-intra cellular parasitic agent, and other treatments for various compounds and combinations as described.

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DHEA Combination Therapy

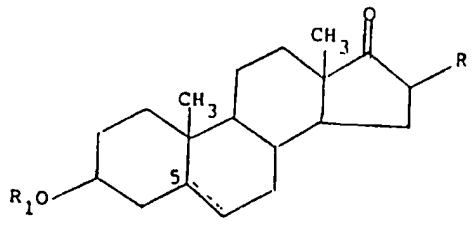
SUMMARY OF THE INVENTION

According to the invention, there are provided combinations of compounds for use in restoring normal levels of Interleukin 12 (IL-12) and/or Interleukin 10 (IL-10) by enhancing or curtailing synthesis or effect of Interleukin 12 and/or Interleukin 10.

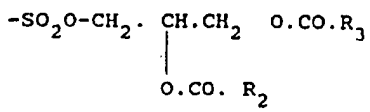
In one aspect of the present invention, the present inventor has found that the anti-viral agents (general formula I herein) as disclosed in U.S. Patent No. 4,956,355 (Prendergast) have additional beneficial therapeutic effects when used in a combination therapy with agents that inhibit Interleukin 10 synthesis and/or action. Agents which inhibit Interleukin 10 can be identified by identifying those compounds which have the ability to inhibit cyclic AMP activity in addition to agents which demonstrate Interleukin 10 inhibition when employed in the screening protocol (Screening IL-10) as herein described. The synthesis of Interleukin 10 can be inhibited by any of a variety of compounds, including one or a combination of the following compounds: - Canavanine Sulphate, L-Canavanine Sulphate, Herbimycin A (Wako Pure Chemicals Industries, Ltd., Japan), Genistein (Sigma Chemicals Co., St. Lous, Mo., USA), secalonic acid D, isoflavinoids, cytokinins, amphiphilic triterpenoids, or analogues to the above together with polyclonal or monoclonal antiserum to Interleukin 10 or any of its peptide sequences.

The anti-viral agent is a 17-ketosteroid compound having the general formula (I)

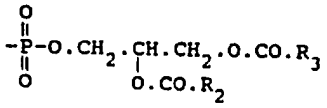
(I)



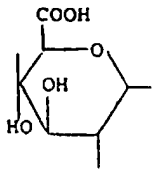
in which R is a hydrogen atom, and R_1 is a chemical group selected from the group consisting of a hydrogen atom, an SO_2OM group wherein M is selected from the group consisting of a hydrogen atom, a sodium atom,
a sulphatide group



wherein each of R_2 and R_3, which may be the same or different, is selected from the group consisting of straight and branched chain alkyl radicals of 1 to 14 carbon atoms,
a phosphatide group



wherein each of R_2 and R_3, which may be the same or different, is selected from the group consisting of straight and branched chain alkyl radical of 1 to 14 carbon atoms, and a glucuronide group



wherein the broken line represents an optical double bond, and the hydrogen atom at position 5 is present in the α- or β- configuration, or the compound comprises a mixture of both configurations. When R_1 is other than a hydrogen atom, the compounds are conjugated compounds.

In another aspect of the present invention, there is provided a method of enhancing the Th_1 immune protective response when using one or more 17-ketosteroid compound as an anti-viral, anti-bacterial, anti-mycoplasm or anti-intra cellular parasitic agent by combining it with anti-serum either poly or monoclonal to Interleukin 10 (cytokine inhibitory factor) and/or with any compound which can effectively inhibit synthesis or the biological function of this specific cytokine Interleukin 10 and/or an Interleukin 10 (cytokine inhibitory factor) receptor molecule blocking agent.

For example, Th_1 immune protective response is required by patients in need of anti-cancer, anti-viral, anti-metastatic, anti-multi drug resistant cancer cell and/or bacterial, non-resistant bacterial infection therapy.

The present invention is also directed to the use of such compounds in the manufacture of a medicament for providing any such treatment.

The pharmaceutical formulation according to the invention may be administered locally or systemically. By systemic administration is meant any mode or route of administration which results in effective levels of active ingredient appearing in the blood or at a site remote from the site of administration of said active ingredient.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulation may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin capsules, dragees, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

Solid dosage forms in addition to those formulated for oral administration include rectal suppositories.

Suitable formulations for topical administration include creams, gels, jellies, mucilages, pastes and ointments. The compounds may be also be formulated for transdermal administration, for example, in the form of transdermal patches so as to achieve systemic administration.

Suitable injectable solutions include intravenous, subcutaneous and intramuscular injectable solutions. The compounds may also be administered in the form of an infusion solution or as a nasal inhalation or spray.

The pharmaceutical formulation according to the invention is administered in unit doses comprising from 10 to 1000 mg of active ingredient. Preferably, each unit dose comprises from 5 to 500 mg of each active ingredient. As per this invention the pharmaceutical formulation contains at least two active ingredients.

According to one embodiment of the invention, the combination therapy is administered at a rate of from 1 unit dose to 10 unit doses per day. Administration of the therapy in accordance with the invention is continued for a period of at least one day and in certain cases may be given for the life of the individual.

Compounds according to general formula (I) are disclosed in U.S. Patent No. 4,956,355 (Prendergast) the entirety of which is hereby incorporated by reference.

Preferably in the compound of formula (I), R and R_1 are each hydrogen. An especially preferred compound is dehydroepiandrosterone (DHEA) wherein R and R_1 are each hydrogen and the double bond is present.

In a further embodiment of the invention, the compound is epiandrosterone wherein R and R_1 are each hydrogen and the double bond is absent. This unsaturated 5-position steroid can also be prepared as an anti-viral agent wherein the R position is occupied by any of the following halogens (bromine, chlorine, fluorine, iodine).

In a further embodiment of the invention, the compuond is 16α-bromoepiandrosterone, wherein R is Br, R_1 is H and the double bond is present. In a still further embodiment of the invention, the compound is according to formula I, wherein R is Br, R_1 is H and the double bond is not present (i.e., where the dotted line is shown in formula I, there is a single bond).

Other preferred compounds are dehydroepiandrosterone sulphate, wherein R is H, R_1 is SO_2-OM and M is as hereinbefore defined and the double bond is present, and 5β-androstan-3β-ol-17-one.

Alternatively, the compound is selected from dehydroepiandrosterone sulphatides, phosphatides or glucuronide wherein R is H, and R_1 is a sulphatide, phosphatide, or glucuronide group as hereinabove defined, and the double bond is present. In particular, when R_1 is not hydrogen, the compounds are DHEA conjugates such as hexyl sulfate, dodecyl sulfate, octadecyl sulfate, octadecanoylglycol sulfate, O-dihexadecylglycero sulfate, hexadecane sulfonate, dioctadecanoylglycero phosphate, O-hexadecylglycero phosphate.

Houston Study

Experimental evidence using DHEA therapy in HIV+ patients has demonstrated that IL-12 levels, as measured by antibody ELISA methods, are elevated, natural killer cell levels increased together with the synthesis and presence of γ (gamma) interferon, HIV viral loads as measured by HIV PCR (RNA) measurement and quantitative culturing techniques demonstrated more than one log reduction after four weeks of DHEA monotherapy. However, while viral load levels were considerably reduced, Th_1 immune improvement did not occur. In fact, due to the elevated levels of Interleukin 12 generated by this monotherapy with DHEA, Interleukin 10 levels increased, which caused a subsequent decline in T4 (helper) cell numbers and the disappearance of the Th_1 (Delayed Type Hypersensitivity Response). Skin reaction in patients as evidenced by patient data was down-regulated by DHEA monotherapy, contrary to previous beliefs of some. Skin reaction is only restored by the removal of Interleukin 10 which is elevated by the DHEA monotherapy.

San Francisco Study (In-vivo)

The following is a summary of using DHEA as a monotherapy in an open-label dose-escalation trial of oral DHEA (Dehydroepiandrosterone) tolerance and pharmacokinetics in patients with HIV disease. In the Phase

I DHEA trial (early symptomatic HIV disease and 200 to 500 CD4+ lymphocytes/μL), absolute CD4 counts in the control, placebo-assigned patients declined by a median 5 cell/month. In contrast, patients in the lowest-dose group studied in the Phase I DHEA trial (whose immune system would not be expected to decline faster than that of placebo-treated patients in the other trial) had a median CD4+ decline of 31 cells/month.

In-Vivo Trial using Combination Therapy

To counteract this Th_1 suppressive immune side effect of DHEA monotherapy this anti-viral steroid had to be combined with an agent to inhibit or interrupt the synthesis and/or action of Interleukin 10. This combination therapy is the preferred embodiment of using the said anti-viral agents (compounds according to general formula I) wherein the anti-viral agents are allowed to generate a Th_1 response. The component of the combination therapy which counteracts the Th_1 suppressive Interleukin 10 immune side effect of the anti-viral therapy may be anti-serum either polyclonal or monoclonal in origin to Interleukin 10 and/or compounds to inhibit or interrupt the synthesis or effectiveness of the unwanted Interleukin 10. Representative compounds which inhibit Interleukin 10 are disclosed in U.S. Patent No. 5,292,725 (Prendergast), the entirety of which is hereby incorporated by reference, which may be used in the combination therapy to counteract the Th_1 suppressive immune side effect of the anti-viral monotherapy.

When the combination therapy was administered to HIV+ patients, the removal of viral particles from each patient's bloodstream was enhanced by 3 (three) logs, relative to the monotherapy, while simultaneously enhancing by over 80% the Th_1 (T4 helper cell count). The Delayed Type Hypersensitivity response lost at sero-conversion was also restored. This combination therapy using DHEA as the non-toxic, non-resistant strain development anti-viral agent, combined with antiserum and/or compounds necessary to inhibit Interleukin 10 synthesis, and/or effect of Interleukin 10, allows for substantial therapeutic benefit to be achieved which previously could not be accomplished by the use of DHEA alone as a monotherapy. The

beneficial action of up-regulation of the immune system together with the anti-viral action of compounds of formula I (U.S. Patent No. 4,956,355--Prendergast) would have wider therapeutic usefulness than in HIV treatment.

Discussion

The aspect of cytokine production following the administration of DHEA has allowed us to review the therapeutic benefits previously ascribed to DHEA therapy. We now know that the therapeutic benefit of DHEA therapy to lupus patients and to other Th_1 auto-immune conditions is directly related to the increase of endogenous Interleukin 10 levels achieved in the patient by the administration of DHEA. Bone marrow transplant rejection was put into remission by DHEA administration to enhance IL-10 levels.

Doctor's Report

Patient: RD - DOB 14/7/1983

RD is a patient under my care. He has Acute Myeloid Leukaemia M3 in remission following allogenic bone marrow transplant. His major active problems have been GUT Graft Versus Host Disease and severe lung disease. RD's general health has improved over the last 3 months. This has coincided with him taking the therapy and he is now enjoying good health. For the first time since his diagnosis he has been able to enjoy full days at school. He no longer needs nasal gastric feeds or suffers with diarrhea. His lung function remains at 30% but his exercise tolerance has improved dramatically. He no longer needs a wheelchair and can tolerate light exercise. As he is on no other drug regime and has been taking this medication for nearly 3 months we must consider that this therapy is influencing these beneficial effects on his body. Before commencing the therapy he was nebulizing Ventolin, Atrovent and Pulmicort four times per day, with oral steroids when necessary. Now he nebulizes only twice a day. I have never seen such a vast improvement in his health with no apparent side effects.

Patients in our experiments who have achieved elevations of their endogenous Interleukin 10 levels have experienced remission in lupus

whereas patients who have been administered DHEA, but who, due to other cytokine and immune factors, have not experienced an Interleukin 10 elevation have not demonstrated relief of symptoms. Therefore, we have identified that a more direct means of causing immediate relief in these autoimmune conditions is to administer exogenously recombinant Interleukin 10 to facilitate the remission of symptoms of lupus and graft versus host disease.

Another area of potential therapeutic benefit previously ascribed to DHEA is the enhancement of vaccine antigen recognition by the immune system in the elderly. This has now been identified and verified by our analysis of the Interleukin 10 levels achieved versus the DHEA therapies efficacy as vaccine adjuvant. We have identified that the administration of recombinant Interleukin 10 to the elderly in association with or in advance of treatment with an antigen vaccine created an enhanced adjuvant effect, which enhanced the antibody response directly. Whereas with DHEA there is a chance of not producing elevated levels of Interleukin 10. The effectiveness of DHEA therapy really depends on the metabolism, blood levels achieved and timing of the DHEA administration to the patient with or prior to the vaccine antigen. The co-administration of recombinant Interleukin 10 is a more direct means of achieving enhanced antibody response in the elderly or very young patient and removes the uncertainties of steroid metabolism and cytokine responses associated with DHEA monotherapy. This for the first time explains why DHEA has very often produced contradictory responses in the therapy of specific conditions e.g., Lupus, MS and HIV because the real therapeutic effect is dependent upon cytokine profiles and immune reactions which are generated upon the administration of the steroid or its analogues. Therefore, the therapeutic effectiveness of DHEA is unpredicatable as an immune modulator and dependent upon both the steroids metabolism and the cytokine profile of the patient during and prior to DHEA therapy. When the steroid is first administered the immune therapeutic response is very much patient specific and cannot be relied upon to produce

consistent therapeutic benefit. Desired immune therapeutic response can only be achieved by utilizing directly the Interleukin 10 cytokine required or by co-administering Interleukin 10 inhibitors and/or specific antisera to same. DHEA thus administered in a combination therapy will facilitate Interleukin 12 enhancement without the general negative effects of Interleukin 10 whereas Th_1 response is desired for therapeutic benefit. Experiments with DHEA and the cytokines profile of patients who respond to DHEA therapy and those who do not respond to DHEA therapy have led to my discovery that elevated Interleukin 10 is the active agent responsible for the therapeutic response observed to alleviate the clinical symptoms of lupus. Other experiments with DHEA and cytokine profiles of senior patients, who responded to a vaccine antigen with enhanced antibody production, has led to the discovery that Interleukin 10 is the active agent responsible for creating the enhanced vaccine response. In general this patient profile would normally, due to age, have reduced antigen vaccine take or immune response. Multiple Sclerosis is a Th_1 auto-immune condition and requires Interleukin 10 to down regulate the Th_1 immune response and cause remission of the condition. We have found a similar response to DHEA therapy for this condition, as we discovered with lupus, i.e., great patient variability to treatment. Any remission of symptoms in Multiple Sclerosis was identified by patients who experienced significant elevation of their endogenous levels of Interleukin 10. Therefore, direct admission of recombinant Interleukin 10 to a multiple sclerosis model in the Lewis rat demonstrated remission of symptoms. If Interleukin 10 is administered prior to the onset of myelin damage the symptoms would be prevented altogether.

<u>Washington in-vitro DHEA IL-12 Study with HIV+ blood</u>

<u>Protocol to demonstrate that DHEA enhances endogenous levels of Interleukin 12</u>

Restoration of HIV-Specific Cell-Mediated Immune Responses by DHEA

One HIV-1 negative control (E9B) and three HIV-1 positive specimens (E9C, E9E and E9F) were stimulated by the addition of DHEA or IL-12 in the

presence of gp120. The stimulation caused by the DHEA in each of these cases was equal to or greater than that caused by the IL-12, although the concentration of DHEA causing the stimulation varied from sample to sample. In the remaining blood samples, E9A (HIV-1 negative) as well as E9D and E9G (HIV-1 positive) proliferation in the presence of gp120 was suppressed by the addition of DHEA or IL-12.

Human IL-12 is a disulfide-bonded heterodimeric cytokine consisting of a 40- and a 35-kD subunit. The genes for this cytokine have been cloned and purified recombinant protein has been produced. It has recently been demonstrated that in vivo administration of murine Interleukin 12 (IL-12) to mice results in augmentation of cytotoxic natural killer (NK)/lymphocytes-activated killer cell activity, enhancement of cytolytic T cell generation, and induction of interferon gamma secretion. In this study, the in vivo activity of murine IL-12 against a number of murine tumors has been evaluated. Experimental pulmonary metastases or subcutaneous growth of the B16F10 melanoma were markedly reduced in mice treated intraperitoneally with IL-12, resulting in an increase in survival time. The therapeutic effectiveness of IL-12 was dose dependent and treatment of subcutaneous tumors were effectively treated by IL-12 at doses which resulted in no gross toxicity. Local peritumoral injection of IL-12 into established subcutaneous Renca tumors resulted in regression and complete disappearance of these tumors. IL-12 was as effective in NK cell-deficient beige mice or in mice depleted of NK cell activity by treatment with antiasialo GM1, suggesting that NK cells are not the primary cell type mediating the antitumor effects of this cytokine. However, the efficacy of IL-12 was greatly reduced in nude mice, suggesting the involvement of T cells. Furthermore, depletion of CD8+ but not CD4+ T cells significantly reduced the efficacy of IL-12. These results demonstrate that IL-12 has potent in vivo antitumor and antimetastatic effects against murine tumors and demonstrate as well the critical role of CD8+ T cells in mediating the antitumor effects against subcutaneous tumors.

Los Angeles Patient Study

The involvement of Interleukin 12 with CD8+ cell generated was demonstrated in a HIV+ patient study conducted for this patent. Patients with a CDE8+ cell population showed an 84% increase above baseline values and IIIV viral load was reduced to zero by the administration of polyclonal antibodies to human Interleukin 10. The removal of Interleukin 10 allowed CD8+ cell increase and allowed for HIV viral clearance by restoring HIV specific cell mediated Immune response.

Specification of Polyclonal Antiserum

PRODUCTION SPECIFICATIONS

Description:	Rabbit anti-Human IL-10
Form:	Liquid
Concentration:	2.7 mg/ml
Stabilizers:	None
Preservative:	None
Sterility:	Sterile filtered
Host Species:	Rabbit
Antibody Class:	IgG
Antigen Used:	Recombinant human IL-10
Method of Purification:	Ion Exchange chromatography
Method of Quantification:	Pierce BCA Protein Assay
Specificity:	Human IL-10
Cross-Reactivity:	No cross reactivity with WHO standards: IL-1a, IL-1B, IL-2, IL-3, IL-4, IL-6, IL-7 IL-8, MIP-1a, TNFa and GM-CSF done by EIA.
Storage:	Short term 4°C and -20°C for long term

Material and Reagents: used to demonstrate DHEA's ability to enhance Interleukin 12 synthesis.

1. IL-2 ELISA, available in house, minimum of six plates.
2. MTS assay, Promega, minimum of 7 plates.
3. IL-12 R&D Systems, (#219-IL) 5 fg should be sufficient for the entire experiment.

4. Antibody to human IL-2 receptor, R&D Systems (AB-233-NA), 1 mg lyophilized, goat human.

5. Rabbit polyclonal antibody to p40 chain of human IL-2, Genetics Institute, Cambridge, MA (617-498-8647).

6. Native gp 120, available in house (50 fg/vial, about 1 mg/mL). Need 5nM/mL. 50 fg is enough for two assays with two plates each.

7. Normal human (HIV-1 negative) PBMC unstimulated.

8. 5 HIV+ samples of blood from which to obtain non-responsive PBMC. 5 mL per sample.

9. DHEA (dehydroisoandrosterone), Sigma D4000. 1 g should be sufficient for the entire experiment.

10. 100% Ethanol to solubilize the DHEA.

11. R10 Medium: RPMI, 10% FBS, 50 fg/mL gentamicin.

12. 96 well flat bottom tissue culture grade cluster dishes, 2 per blood sample.

Protocol:

1. For each blood sample, separate out PBMCs and do a cell count.

2. Use all the cells available from patient samples. If 10×10^6 cells or more are present, seed the cells into two 96 well plates. At 10×10^6 we will end up with 0.5×10^5 cells/well or 2.5×10^5 cells/mL. If fewer use only one plate. Record the number actually plated per well. If two plates are used one will be for IL-2 detection and will receive antibody to human IL-2 receptor. The other plate will be used for the cell proliferation assay and will not receive this antibody. If only one plate is used, that plate will receive antibody.

3. If using one plate resuspend the cells in 20 mL R10; if two plates resuspend in 40 mL. Aliquot 200 fL per well. Allow to settle overnight. If natural settling is not practical, wrap plates in plastic wrap and use gentle centrifugation.

4. Prepare schema showing which special media will be added to which wells. (Be aware that due to the need for blanks and standards needed for the IL-2 ELISA not all replicates grown up will be used in the ELISA).

5. Each experiment will need 16 or 32 mL of medium with native gp 120 at 5 nM/mL. FW = 120,000. Amount to add per 16 mL; 96 fL of a 100 fg/mL stock. (6 fL of 100 fg/mL for each mL of medium).

Also use 12 or 24 mL of R10 medium for each assay.

Note well: This will be the key to whether the PBMC are reactive or not. If the cells proliferate and produce IL-2 in the presence of gp 120 and not without gp 120 these cells are normal reactive cells. If they behave the same vis-a-vis proliferation and IL-2 production regardless of whether gp 120 has been added, the cells are non-reactive. It is only in the non-reactive cells that we should see the effect of IL-12 and DHEA.

6. To the 16 mL with gp 120 and the 12 mL of R10 for each sample to be used for the IL-2 ELISA, add 2 fg/mL of antibody to the IL-2 receptor.

7. Preparation of DHEA:

7.1 Dissolve 1 g of DHEA in 1 mL of absolute ethanol (100%). Incubate in a 37°C water bath. Additional ethanol may be added up to 3.47 mL. This will give a 1 M solution. If the entire 3.47 mL is not needed for it to go into solution the difference can be made up with R10 medium.

7.2 For each sample, we will need with medium DHEA at the following concentrations: 10^{-4}, 10^{-6}, 10^{-10}, 10^{12}.

7.3 At each dilution prepare 2 mL of media (already containing gp 120 and antibody from step 6) and another 2 ml of media with gp 120 but no antibody if a second plate is used. For 10^{-8}, 6 mL of each will be needed.

At each dilution prepare 2 x 1.5 mL of R10 medium without gp 120 and with and without antibody from step 6. For 10^{-8}, 3 mL of each will be needed.

7.4 Making dilutions. Use 5 mL tubes.

A. Take 20 fL of 1 M DHEA into 2 mL of R10 medium = 10^{-2}M.

B. Take 20 fL of 10^{-2} M DHEA into 2 mL of step 4.3 medium = 10^{-4}M.
Take 15 fL of 10^{-2} M DHEA into 1.5 mL of R10 = 10^{-4}M.

C. Take 20 fL of 10^{-4} M DHEA into 2 mL of step 4.3 medium = 10^{-6}M.
Take 15 fL of 10^{-4}M DHEA into 1.5 mL of R10 = 10^{-6}M.

D. Take 40 fL of 10^{-6} M DHEA into 4 mL of step 4.3 medium = 10^{-8}M. Take 30 fL of 10^{-6}M DHEA into 3 mL of R10 = 10^{-8}M.

E. Take 20 fL of 10^{-8} M DHEA into 2 mL of step 4.3 medium = 10^{-10}M. Take 15 fL of 10^{-8} M DHEA into 1.5 mL of R10 = 10^{-10}M.

F. Take 20 fL of 10^{-10} M DHEA into 2 mL of step 4.3 medium = 10^{-12}M. Take 15 fL of 10^{-12}M.

7.5 To half of the 10^{-8} M DHEA media types add antibody to human IL-12.

7.6 IL-12 medium for each plate:

A. To 2 ml of gp 120 medium with and without antibody for IL-2 add 10 U/mL of recombinant IL-12.

B. To 1.5 mL of R10 with and without antibody to IL-2 add 10 U/mL of recombinant IL-12.

C. 1 ED_{50}=1U. The ED_{50} of the IL-12 will be in the literature received with this reagent.

8. Aspirate medium off of cells and add 200 fL of appropriate medium to each well according to the schema. Place extra medium in peripheral wells. Wrap plates in plastic wrap and place on tray with water. Incubate at 37°C, 5% CO_2.

9. If it is a two plate assay, after 5 days aspirate off medium from the plate without antibody to the IL-2 receptor. Replace with 100 fL/well of R10 medium. Perform the cell proliferation assay with a 4h incubation.

10. After 7 days using the plate with the antibody to the IL-2 receptor: Take 100 fL per well and use to perform the IL-2 ELISA.

11. If there is only one plate for an assay, remove and freeze the rest of the supernatant from each well, then add 100 fL/well of R10 medium and proceed with the cell proliferation assay at this 7 day point.

12. Run PBMC from HIV- blood first to see if all reagents are performing as expected before proceeding with HIV+ samples.

13. Another HIV- sample should be run after all the HIV+ samples have been completed.

14. Compile and analyze data.

Protocol Summary

Title: A Clinical Trial of Administered DHEA combined with Isopentenyl adenosine 5'-monophosphate as a specific inhibitor of Interleukin 10 Specially Formulated for Persons with HIV infection who have developed resistance to protease and RT inhibitors.

DHEA combined with Isopentenyl adenosine 5'-monophosphate herein referred to as Compound (D+I).

Indication: Treatment of HIV-1 infection.

Type of Study: Phase I/II Clinical Trail.

Study Objectives:

a. Determine the safety and tolerance of administered Compound (D+I) in persons with advanced HIV diseases.

b. Determine the effect of administration of Compound (D+I) on measures of HIV Viral Load. Serum PCR (RNA) levels together with HIV p24 antigen (by acid dissociation method).

c. Determine the immune and toxicological effects of administered Compound (D+I).

d. Determine the pharmokinetics of administered Compound (D+I).

Inclusion Criteria

a. Age 18 years or older;

b. HIV-1 seropositive;

c. A CD4+ -T-lymphocyte count of 50 to 300 cells/mm^3 within one month prior to study entry, measured on two separate occasions 72 hours to 28 days apart;

d. The following baseline laboratory values:

Hemoglobin > 9g/dl

WBCs > 1500 cells/µl

Neutrophils > 1000 cells/µl

Platelets 25,000 cells/µl

Bilirubin <2.0mg/dl

AST, ALT, Alkaline Phosphatase <5x upper limit of normal

Creatinine <1.5 mg/dl;

e. A history of prior anti-retroviral therapy as follows:

i. In patients with a prior history of anti-retroviral therapy using AZT, ddI, ddC or d4T alone or in combination with protease inhibitors who are not receiving such therapy at study entry, these patients must have discontinued this medication at study entry.

g. Use of suitable contraception by women of childbearing potential (requires one negative serum pregnancy test, beta-HCG, within one week prior to study entry in women of childbearing potential).

h. Medium to high PR HIV RNA titre at entry to study.

Exclusion Criteria:

a. Previous treatment with chemotherapeutic agents within eight weeks of enrollment;

b. Active, major infection, including AIDS-defining opportunistic infection, or other life-threatening medical crisis;

c. Pregnant or breast-feeding;

d. Any condition which, in the investigator's opinion places the patient at undue risk or jeopardized the objectives of the trial;

e. Receiving immunomodulatory therapies including interferon or pharmacological doses of steroids at entry into the study;

Safety Measures: Weekly analysis up to week 4 of the study of the following parameters:

i. Documentation and assessment of adverse events.

ii. Hematology.

iii. Clinical chemistries and urinalysis.

iv. Assessment of the immune responses resultant from Compound (D+I).

v. Assessment of PCR (RNA) and DNA measures alteration with therapy.

Effectiveness Measures:

Measures of viral load will include HIV-p24 antigenemia, and HIV-RNA PCR (cell free, serum) and cell HIV-DNA analysis.

Improvements in immune response will be measured as changes from baseline in CD4/CD8 ratio. Clinical lymphocyte counts, percent alterations in WBC, in Interleukin 10 levels which would demonstrate the ability of Compound (D+I) to cause the patients' immune system to move to T_H-1 status.

Clinical benefit will be assessed by change in total body weight, Karnofsky performance score, and amelioration of signs and symptoms of disease present at baseline.

The remission or incidence of new opportunistic infection will be summarized.

Study Design:

Open-label, daily administration of a dose per patient of 1200 mg/day, with review and assessment of the dosage schedules and efficacy after therapy for 4 weeks.

Study Size: 5 Patients (total) - 5 patients @ 1200 mg/day for 30 days.

Test Articles:

Test Drug: Compound (D+I) particle size distribution, 87%: < 5 µm, 100%: <15 µm, administration in gelatine capsules of 200 mg per capsule. Each capsule contains: 600 mg of DHEA and 600 mg of Isopentenyl adenosine 5'-monophosphate

Control Drug: None

Placebo: None

Patient data required before and after administration of Compound (D+I)

	Screen	Week				
	Base-line	1	2	3	4	8
Physical Exam & Medical History	X	X	X	X	X	
Urinalysis	X	X	X	X	X	
Glucose	X	X	X	X	X	
Neopterin	X	X	X	X	X	
Beta2-microglobulin	X	X	X	X	X	
RBC	X	X	X	X	X	
hb	X	X	X	X	X	
WBC	X	X	X	X	X	X
Platelet	X	X	X	X	X	X
T Cell Panel	X	X	X	X	X	X
p24 Antigen	X	X	X	X	X	
Creatinine	X	X	X	X	X	
SGOT	X	X	X	X	X	X
SGPT	X	X	X	X	X	X
IgG	X	X	X	X	X	
IgA	X	X	X	X	X	
IgM	X	X	X	X	X	
DHEA	X	X	X	X	X	X
DHEAS	X	X	X	X	X	X
Testosterone	X	X	X	X	X	X
17 Ketosteroids	X	X	X	X	X	X
Interleukin 10	X	X	X	X	X	X
Interleukin 2	X	X	X	X	X	X
PCR (RNA) (Cell free, serum)	X	X	X	X	X	X
PCR (DNA)	X	X			X	X
CD4	X	X	X	X	X	X

I Claim

1. A method of enhancing the Th_1 immune protective response when using one or more 17-ketosteroid compound as an anti-viral, anti-bacterial, anti-mycoplasm or anti-intra cellular parasitic agent by combining it with anti-serum either poly or monoclonal to Interleukin 10 (cytokine inhibitory factor), or with any compound which can effectively inhibit synthesis or the biological function of this specific cytokine Interleukin 10 whose synthesis is detrimentally enhanced by DHEA monotherapy, or with an Interleukin 10 (cytokine inhibitory factor) receptor molecule blocking agent.

2. A method of enhancing the Th_2 immune response when using one or more 17-ketosteroid compound as an anti-viral, anti-bacterial or anti-intra cellular parasitic agent by combining it with anti-serum either poly or monoclonal to Interleukin 12, or with any compound which can effectively inhibit synthesis or the biological function of this specific cytokine Interleukin 12 whose synthesis is detrimentally enhanced by DHEA monotherapy during the course of Th_1 activated disease state, or with an Interleukin 12 receptor molecule blocking agent.

3. A method of enhancing the Th_2 immune response when using one or more 17-ketosteroid compound as an anti-viral, anti-bacterial or anti-intra cellular parasitic agent by combining it with anti-serum either poly or monoclonal to Interleukin 2, or with any compound which can effectively inhibit synthesis or the biological function of this specific cytokine Interleukin 2 whose synthesis is detrimentally enhanced by DHEA monotherapy during the course of a Th_1 activated disease state, or with an Interleukin 2 receptor molecule blocking agent.

4. A method of enhancing the Th_1 immune protective response when using one or more 17-ketosteroid as an anti-viral, anti-bacterial, anti-mycoplasm or anti-parasitic agent by combining it with anti-serum either polyclonal or monoclonal to human alpha-fetoprotein or similarly immunosuppressive peptides of human bacterial, viral or synthetic origin.

5. A method as recited in any one of claims 1-4, wherein the Th_1 immune protective response is required by a patient in need of anti-cancer, anti-viral, anti-metastatic, anti-multi drug resistant cancer cell and/or bacterial, non-resistant bacterial infection therapy.

6. A method as recited in claim 1, wherein polyclonal and/or monoclonal anti-serum is used to remove or neutralize Interleukin 10 and is directed to the Epstein-Barr virus open reading frame BCRF1 which has amino acid sequence homology to human cytokine synthesis inhibitory factor (Interleukin 10).

7. A process of administering to a patient a combination therapy of at least one 17-Ketosteroid with an Interleukin 10 inhibitor or an Interleukin 10 receptor molecule blocking agent.

8. A process of administering to a patient a combination therapy of at least one 17-Ketosteroid with an Interleukin 12 inhibitor or an Interleukin 12 receptor molecule blocking agent.

9. A process of administering to a patient a combination therapy of at least one 17-Ketosteroid with an Interleukin 2 inhibitor or an Interleukin 2 receptor molecule blocking agent.

10. A method of treating or arresting the progression of an immune dysfunction in a patient in need of such treatment which comprises administering a combination therapy of at least one 17-Ketosteroid with an Interleukin 10 Inhibitor or an Interleukin 10 receptor molecule blocking agent.

11. A method of treating or arresting the progression of an immune dysfunction in a patient, in need of such treatment, which comprises administering a combination therapy of at least one 17-Ketosteroid with an Interleukin 12 inhibitor or an Interleukin 12 receptor molecule blocking agent.

12. A method of treating or arresting the progression of an immune dysfunction in a patient in need of such treatment which comprises administering a combination therapy of at least one 17-Ketosteroid with an Interleukin 2 inhibitor or an Interleukin 2 receptor molecule blocking agent.

13. A process of administering to a patient recombinant or cloned Interleukin 10 for the therapy of Lupus and/or Graft versus host disease.

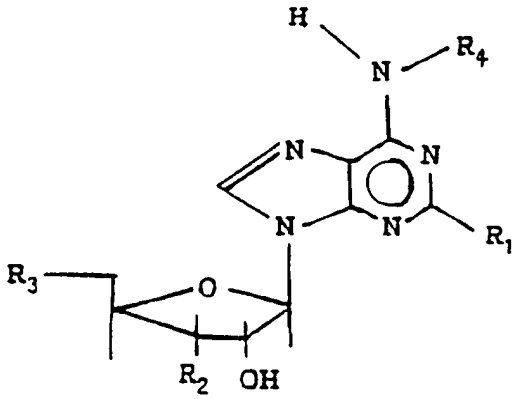
14. A process of administering to a patient recombinant or cloned Interleukin 10 as adjuvant in vaccine therapy.

15. A process of treating multiple sclerosis in a patient in need of such treatment, comprising administering recombinant or cloned Interleukin 10 to said patient.

16. A method of treating Lupus or graft versus host disease in a patient in need of such treatment, comprising administering recombinant or cloned Interleukin 10 to said patient.

17. A method as recited in claim 1, 7 or 10 wherein the synthesis of Interleukin 10 is inhibited by any one or a combination of the following compounds: - Canavanine Sulphate, L-Canavanine Sulphate, Herbimycin A, Genistein, secalonic acid D, isoflavinoids, cytokinins, amphiphilic triterpenoids, or analogues to any of the above.

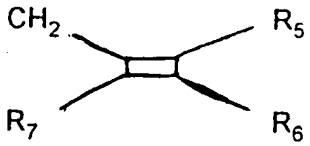
18. A method as recited in claim 17 wherein the cytokinin is selected from the group having the formula



wherein:

R_1 = H, R_2 = CH_3, R_3 = CH_3 and R_4 = H, or

R_1 = H or CH_3S and

R_4 = 

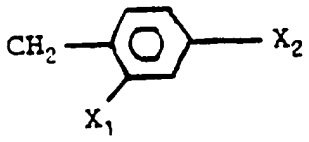
and

R_5 = CH_3, Cl, OH or a monophosphate group

R_6 = CH_3, CH_2OH or Cl

R_7 = H or Br

or R_1 = H and

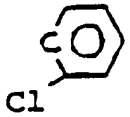
R_4 = 

and X_1 and X_2 are independently selected from H, methyl, ethyl, hydroxyl, the halogens and carboxyl

or R_4 = 

or R_4 = 

or R_4 = $\overset{O}{\overset{\|}{C}}NH\text{-}R_8$

and R_8 = 

or $R_8 = (CH_2)_7CH_3$;

and R_2 = OH and R_3 = OH, monophosphate, diphosphate or triphosphate group or R_2 and R_3 are linked to form a 3', 5'-cyclic monophosphate derivative, or a metabolite of said compound, said metabolite being a member of the group consisting of:

N^6-(Δ^2-isopentenyl)adenine:

6-N-(3-methyl-3-hydroxybutylamino) purine;

Adenine;

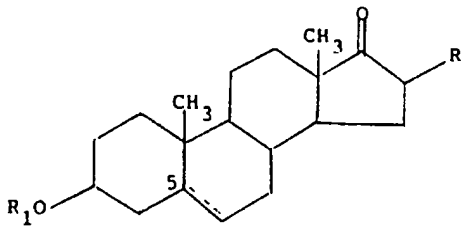
Hypoxanthine;

Uric Acid; and

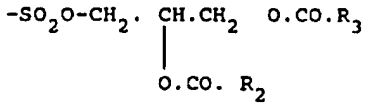
Methylated xanthines.

19. A method as recited in any one of claims 1-12, 17 and 18, wherein said 17-ketosteroid has the formula

(I)

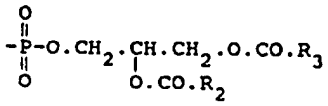


in which R is a hydrogen atom, and R_1 is a chemical group selected from the group consisting of a hydrogen atom, an SO_2OM group wherein M is selected from the group consisting of a hydrogen atom, a sodium atom, a sulphatide group

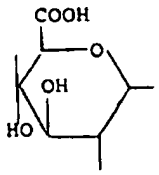


wherein each of R_2 and R_3, which may be the same or different, is selected from the group consisting of straight and branched chain alkyl radicals of 1 to 14 carbon atoms,

a phosphatide group



wherein each of R_2 and R_3, which may be the same or different, is selected from the group consisting of straight and branched chain alkyl radical of 1 to 14 carbon atoms, and a glucuronide group



atom at position 5 is present in the α- or β- configuration, or the compound comprises a mixture of both configurations.

20. A method of treating a viral infection, a bacterial infection, a mycoplasm infection or a parasitic infection in a patient in need of such treatment, comprising administering to said patient:

(1) one or more 17-ketosteroid compound, and

(2) one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 10, compounds which can effectively inhibit synthesis or the biological function of Interleukin 10, and Interleukin 10 receptor molecule blocking agents,

in respective amounts which are effective to provide said treatment.

21. A method as recited in claim 20, wherein said patient is suffering from HIV infection.

22. A method of treating cancer, viral infection, metastasis, multi drug resistant cancer and/or bacterial, non-resistant bacteria in a patient in need of such treatment, comprising administering to said patient:

(1) one or more 17-ketosteroid compound, and

(2) one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 10, compounds which can effectively inhibit synthesis or the biological function of Interleukin 10, and Interleukin 10 receptor molecule blocking agents,

in respective amounts which are effective to provide said treatment.

23. A method of treating Lupus or Multiple Sclerosis and/or Graft versus Host disease in a patient in need of such treatment, comprising administering to said patient recombinant or cloned Interleukin 10.

24. A composition comprising:

(1) one or more 17-ketosteroid compound, and

(2) one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 10, compounds which can effectively inhibit synthesis or the biological function of Interleukin 10, and Interleukin 10 receptor molecule blocking agents.

25. A kit comprising:

(1) at least one unit-dosage of a 17-ketosteroid compound, and

(2) at least one unit-dosage of one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 10, compounds which can effectively inhibit synthesis or the biological function of Interleukin 10, and Interleukin 10 receptor molecule blocking agents.

26. A method of treating Lupus or Multiple Sclerosis and/or Graft versus Host disease in a patient in need of such treatment, comprising administering to said patient recombinant or cloned sequences derived from sequences present in the Interleukin-10 molecule or sequences which mimic Interleukin-10's immunosuppressive action.

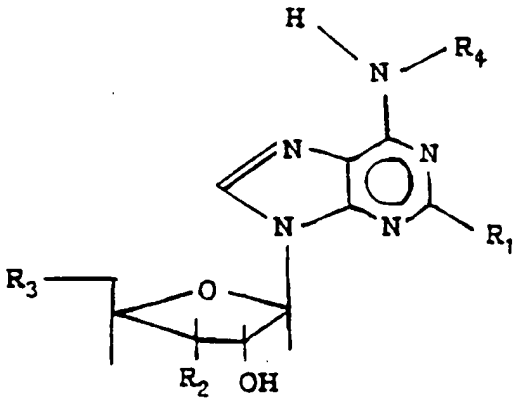
27. A method of treating cancer, viral infection, metastasis, multi drug resistant cancer and/or bacterial, non-resistant bacteria in a patient in need of such treatment, comprising administering to said patient a combination comprising:

(1) recombinant or cloned Interleukin-12 and

(2) one or more member selected from the group consisting of anti-serum, either poly or monoclonal, to interleukin 10 compounds which can effectively inhibit synthesis or the biological function of Interleukin 10, Interleukin 10 receptor molecule blocking agents, in respective amounts which are effective to provide said treatment.

28. A method as recited in claim 1, 7 or 10 wherein the synthesis of Interleukin 10 is inhibited by any one or a combination of the following compounds: - Canavanine Sulphate, L-Canavanine Sulphate, Herbimycin A, Genistein, secalonic acid D, isoflavinoids, amphiphilic triterpenoids, or analogues to any of the above.

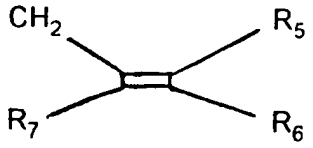
29. A method as recited in claim 1, 7 or 10 wherein the synthesis of Interleukin 10 is inhibited by at least one cytokinin, with the proviso that said cytokinin is other than a compound selected from the group having the formula



wherein:

R_1 = H, R_2 = CH_3, R_3 = CH_3 and R_4 = H, or

R_1 = H or CH_3S and

R_4 = 

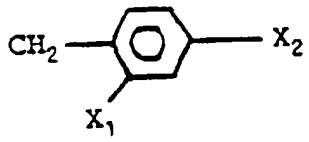
and

R_5 = CH_3, Cl, OH or a monophosphate group

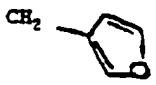
R_6 = CH_3, CH_2OH or Cl

R_7 = H or Br

or R_1 = H and

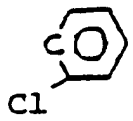
R_4 = 

and X_1 and X_2 are independently selected from H, methyl, ethyl, hydroxyl, the halogens and carboxyl

or R_4 = 

or R_4 = 

or R_4 = $\overset{O}{\overset{\|}{C}}NH\text{-}R_8$

and R_8 = 

or $R_8 = (CH_2)_7CH_3$;

and R_2 = OH and R_3 = OH, monophosphate, diphosphate or triphosphate group or R_2 and R_3 are linked to form a 3', 5'-cyclic monophosphate derivative, or a metabolite of said compound, said metabolite being a member of the group consisting of:

N^6-(Δ^2-isopentenyl)adenine:

6-N-(3-methyl-3-hydroxybutylamino) purine;

Adenine;

Hypoxanthine;

Uric Acid; and

Methylated xanthines.

30. A method of treating lupus or graft versus host disease in a patient in need of such treatment, comprising administering to said patient:

(1) one or more 17-ketosteroid compound, and

(2) one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 12, compounds which can effectively inhibit synthesis or the biological function of Interleukin 12, or Interleukin 12 receptor molecule blocking agents,

in respective amounts which are effective to provide said treatment.

31. A method of treating lupus or graft versus host disease in a patient in need of such treatment, comprising administering to said patient:

(1) one or more 17-ketosteroid compound, and

(2) one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 2, compounds which can effectively inhibit synthesis or the biological function of Interleukin 2, or Interleukin 2 receptor molecule blocking agents,

in respective amounts which are effective to provide said treatment.

32. A method of treating a bacterial infection, a mycoplasm infection or a parasitic infection in a patient in need of such treatment, comprising administering to said patient:

(1) one or more 17-ketosteroid compound, and

(2) one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 12, compounds which can effectively inhibit synthesis or the biological function of Interleukin 12, or Interleukin 12 receptor molecule blocking agents,

in respective amounts which are effective to provide said treatment.

33. A method of treating a bacterial infection, a mycoplasm infection or a parasitic infection in a patient in need of such treatment, comprising administering to said patient:

(1) one or more 17-ketosteroid compound, and

(2) one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 2, compounds which can effectively inhibit synthesis or the biological function of Interleukin 2, or Interleukin 2 receptor molecule blocking agents,

in respective amounts which are effective to provide said treatment.

34. A method for preventing or reducing bacterial translocation in a patient in need of such treatment, comprising administering to said patient:

(1) one or more 17-ketosteroid compound, and

(2) one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 10, compounds which can effectively inhibit synthesis or the biological function of Interleukin 10, or Interleukin 10 receptor molecule blocking agents,

in respective amounts which are effective to provide said treatment.

35. A composition comprising:

(1) one or more 17-ketosteroid compound, and

(2) one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 12, compounds which can

effectively inhibit synthesis or the biological function of Interleukin 12, and Interleukin 12 receptor molecule blocking agents.

36. A kit comprising:

(1) at least one unit-dosage of a 17-ketosteroid compound, and

(2) at least one unit-dosage of one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 12, compounds which can effectively inhibit synthesis or the biological function of Interleukin 12, and Interleukin 12 receptor molecule blocking agents.

37. A composition comprising:

(1) one or more 17-ketosteroid compound, and

(2) one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 2, compounds which can effectively inhibit synthesis or the biological function of Interleukin 2, and Interleukin 2 receptor molecule blocking agents.

38. A kit comprising:

(1) at least one unit-dosage of a 17-ketosteroid compound, and

(2) at least one unit-dosage of one or more member selected from the group consisting of: anti-serum, either poly or monoclonal, to Interleukin 2, compounds which can effectively inhibit synthesis or the biological function of Interleukin 2, and Interleukin 2 receptor molecule blocking agents.

39. A method as recited in claim 1, wherein said Interleukin-10 inhibitor is selected from:

1) NG-monomethyl-l-arginine (L-NMMA), and

1) sodium nitroprusside (SNP).

INTERNATIONAL SEARCH REPORT

Inten nal Application No
PCT/IB 97/00414

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/565 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 355 825 A (PRENDERGAST PATRICK T) 28 February 1990 cited in the application	1,5,7, 10, 17-22, 24,25, 28,29,34
Y	see page 11, line 5-18; claims 1,2,9-12	1,5,7, 10,22, 24,25,34

X	EP 0 405 980 A (SCHERING CORP) 2 January 1991	13-16, 23,26
Y	see page 2, line 48 - page 3, line 12	1,5,7, 10,22, 24,25,34
	see page 10; claims 1,2,15,17,18 & US 5 231 012 A (SCHERING CORPORATION) see column 2, line 42-44	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
9 September 1997	24.09.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Kanbier, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern. al Application No
PCT/IB 97/00414

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 04180 A (SCHERING CORP) 3 March 1994	13-16, 23,26,27
A	see page 1-2; claims 1-4	1,3,5-7, 10,12, 22,24, 25,37,38
	see page 6-7 see page 8, line 21-31 see page 9, line 4-9 see page 10, line 1-5 see page 13, line 28-31 see page 15, line 7 - page 16, line 4 see page 16, line 27-28 see page 17, line 11-18 see page 27, line 12-21 see page 29, line 3-5	

X	US 5 449 688 A (WAHL SHARON M ET AL) 12 September 1995 see column 2, line 11-25; examples 3-5,8	13-16, 23,26
A	see column 2, line 32-44; claims 1-5 see column 3, line 51 - column 5, line 8 see column 6, line 23-42 see column 6, line 53-59	17,28,39

A	JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 26S, 1996, pages 1-28, XP000676752 KELLOFF ET AL: "NEW AGENTS FOR CANCER CHEMOPREVENTION" see page 5, right-hand column - page 6, left-hand column see page 7, right-hand column - page 8, left-hand column see page 2, right-hand column	1,5,7, 10,17, 22,24, 25,28

A	EP 0 340 604 A (CENTRE REGIONAL DE TRANSFUSION) 8 November 1989 see page 2, column 1-3; claims 4,5,7,8	3,9,12, 31,33, 37,38

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INTERNATIONAL SEARCH REPORT

Int tional application No.
PCT/IB 97/00414

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 1-23 26-34 39

because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

Remark : Although claims 1-23, 26-34, 39 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 97/00414

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